REPRODUCTIVE BIOLOGY OF LYCHAS TRICARINATUS (SIMON)[1]

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In a previous paper on the embryonic nutrition in *Lychas tricarinatus* (Mathew, 1960), I had drawn attention to a curious phenomenon of arrest of development in internal embryos in this scorpion. As this phenomenon was an unusual one, further studies were made on it and this paper embodies the results of these investigations.

MATERIALS AND METHODS

Gravid females of *L. tricarinatus* were collected almost every month from Mukkunni hills near Trivandrum (only during the wet season) and from Nagercoil and adjoining hills like Asambu and Thadikarankonam in Kanyakumari District of Madras State. Specimens were also collected from Vaikom and Moovatupuzha in North Travancore. Suitable cages were improvised for them and they were reared in these and observations were made in each case as to the delivery of young. As occasions demanded, the specimens were killed and the conditions of the ovary examined, and all these ovaries were preserved and labelled for future study.

OBSERVATIONS

The ovary consists of a pair of longitudinal tubes on each side, each pair connected together by five transverse tubes forming four quadrilateral meshes on each side; so, unlike other scorpions, here the female system has almost the same arrangement of tubules as in the male system. However, at the hindermost part, the inner longitudinal tubes of either side are fused so that this hind extremity of the ovary alone resembles the ovarian network of other scorpions. The developing embryos are inside the ovarian tubes as typical of the Buthidae. All along the outside of the tubes there are eggs in different stages of growth, in the tiny bubble-like follicles. Among these are a few which are larger than the others. The eggs in them are surrounded by thick envelopes. They have already begun to develop and in some, well developed blastoderms may be seen. Does this mean that while one set of embryos has already begun to develop inside the ovarian tubes, other sets, too, are developing, so that at one time there can be young of different stages of development in the ovarian tube? On morphological and physiological grounds, this is an impossibility and does not happen in any scorpion studied so far. Since

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ovarian tubes themselves are the embryo-ducts, if there are embryos of different stages of development in these tubes, when some of the embryos mature and are extruded, they will push out the younger immature embryos too.

Ovarian tubes in which the embryos are fully mature also have eggs in different stages of growth, in tiny bubble-like follicles, with a few larger follicles enclosing segmenting zygotes or fully formed blastoderms, on the outside; just the same as seen in the earlier stages. Evidently they have remained all through the development of the internal embryos without any change. This would indicate that these zygotes and early embryos outside the ovarian tube are held in arrested state of growth, or in a condition resembling diapause, until the extrusion of the developed embryos. After the extrusion of these developed embryos, the suppressed embryos might get pushed into the ovarian tube and resume their course of development. If this should happen, the scorpion, after giving birth to one brood, must be able to develop another brood of embryos without fresh copulation. To test if this actually happens a few breeding experiments were conducted and observations were made.

BREEDING EXPERIMENT

Six gravid females were selected from a collection from the field and reared in artificial cages providing as nearly natural conditions as possible—one female in a cage. They were properly fed, given insects and water, and observations were recorded.

Female A

Collected and caged	3–5–1958
Delivered the young	9–5–1958
Young moulted	14–5–1958
Young left the mother	19–5–1958

After delivery the mother was kept in the cage and properly looked after. It grew well and after about a month was seen with the abdomen swollen as if it was becoming gravid again. There was no male scorpion anywhere in the vicinity. A little later the abdomen was still more distended and through the thin translucent sterna, the internal embryos could be seen quite clearly. There was no more doubt as to its second pregnancy. On June 20th, *i.e.*, 41 days after the previous extrusion of young, a second brood came out and the young took their position on the mother's back as usual.

Delivery of the second brood	20–6–1958
Young moulted	26–6–1958
Young left the mother	1–7–1958

The female was then transferred to another cage and carefully looked after for another month. By this time it was showing considerable weakness and it was not possible to keep it alive any further. So it was killed on the 28th of July, 1958, and the ovary was examined. There were 27 developing embryos inside, which were fairly advanced and the black pigmented eyes were conspicuous.

With three of the other specimens this experiment was repeated under similar conditions and the above observations were confirmed. Since then these observations were further repeated and confirmed in 1960 and again in 1961 with fresh specimens obtained mostly from Nagelcoil. From these rearing experiments, the following conclusions can be drawn: (1) *Lychas tricarinatus* can, after one delivery, produce at least three successive broods of young when isolated from all possibility of contact with a male. (2) The time elapsing between one delivery and the next is about 40–42 days. This may be taken as the approximate period of gestation. Since the period of gestation can be approximately determined, counting from the time of one delivery, it is possible to keep females in captivity and study the stages of development of embryos within specified periods of time. So a few gravid females were kept in captivity and after one delivery, the embryos were examined and studied at different intervals. In all these there were growing embryos developing at the normal rates, as could be tested by the condition of the embryos at definite periods in the different specimens examined. The development and the stages attained as seen in these studies will be described elsewhere, dealing with the embryology of *Lychas*.

The series of breeding experiments described above show conclusively that this scorpion does produce successive broods without pairing after each brood has been extruded. How are the new embryos formed? The following possibilities may be suggested, but each has to be tested and proved.

(1) It may be that there are slight variations in the developmental stages attained by embryos of a brood in the uterine tube and the most mature are extruded first and the others are retained till they too are fully developed, to be extruded as the second brood, and so on.

(2) Parthenogenesis.

(3) Sperm received at one copulation are preserved in the spermathecae, viable and capable of fertilising eggs for future broods.

(4) After once pairing, numerous eggs are fertilised but only a few of these can develop in the limited space of the uterine tubes (ovarian tubes). These few alone pass into the uterine tubes and continue development while the others are kept in an arrested state of development till the developing brood is extruded.

Regarding the first possibility, suggested above, we may note the following facts and observations: (1) Opening gravid females, we do not notice such disparity in stages of the development of individuals of a brood. In early stages a slight disparity may appear but by the time the embryos have attained about half maturity, all are of the same stage except rarely a diseased or injured individual which fails to be extruded. (2) When a female is opened soon after extrusion of a brood, no younger stages are seen to be retained in the ovarian tubes but they are completely empty and lie flabby in the body cavity; all eggs and zygotes on the ovarian tubes are on the outside only.

Occasionally, when several specimens are opened, one comes across a dead embryo still retained in some part of the ovarian tube. But this is a diseased or dead one which could not be extruded and so has been retained there to undergo gradual histolysis and absorption. These have been observed in other scorpions too, such as *Heterometrus* (Mathew, 1956). This may cause obstruction for the other embryos, but because of the retiform arrangement of the ovarian tubes providing several alternating pathways, no such blockade actually takes place; all viable embryos escape.

These considerations help us to rule out the first possibility.

The second possibility suggested is parthenogenesis. It is known to take place under special conditions in many arthropods, but one indication for parthenogenesis is the absence or rarity of males. With regard to *Lychas,* however, this condition does not hold. Estimating the proportion of the sexes from collections that have been made during these four years, one can say that in number the sexes are almost equal—sometimes there may be a slight predominance of females. At any rate it can be most confidently stated that there is no scarcity of males.

Again, if development is parthenogenetic there is no need for the early formation of zygotes and blastoderms; the development processes may well start after one brood has been extruded.

The third possibility suggested is that viable sperm may be retained in the spermatheca to be used over and over again. This is not an impossibility but microscopic examination of the contents of the seminal vesicles has failed to show sperms held in reserve. Also, as already observed with reference to parthenogenesis, if there is sperm held in reserve for future fertilisation, why the early formation of zygotes and blastoderms?

The last suggestion, which is in agreement with all the observed phenomena, appears to be most satisfactory.

(1) The ovarian tubules of a female which has recently extruded a brood of young possess numerous tiny bubble-like follicles in which there are young ova or zygotes or blastoderms, the latter two being distinguishable by their larger size and being covered over by a thick pigmented shell, the "chorion."

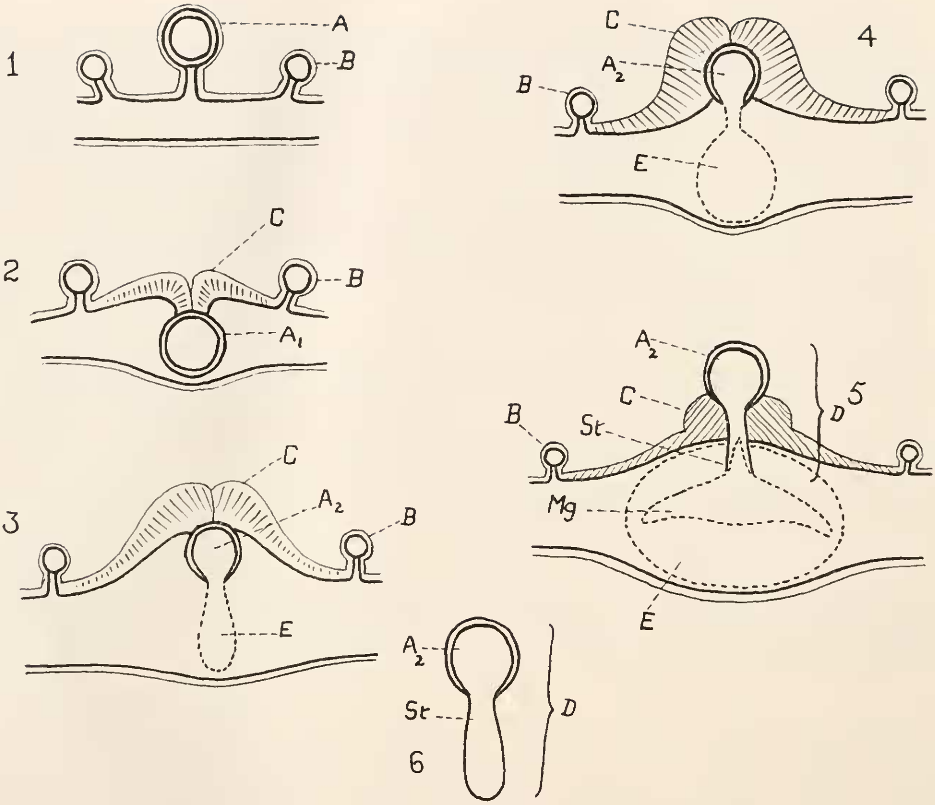
(2) In the ovarian tubes, examined a week later, we find that some of these blastoderms enclosed in the "chorion" have sunk into the ovarian tube while the zygotes that are still outside begin segmentation and develop into blastoderms. Only a limited number of embryos can sink into the ovarian tube and the number appears to be roughly controlled by the extent of space available on the ovarian tube. In an ovarian tube with well developed internal embryos, the swellings are seen more or less evenly spaced, thus giving it the characteristic moniliform appearance.

(3) All through the subsequent development, when the internal embryos are growing to maturity, the external zygotes and blastoderms remain without any further development. They remain dormant till this brood is extruded.

Embryonic Diapause in Lychas tricarinatus

This arrested development described above, as seen in *Lychas tricarinatus,* may be taken to be a special type of diapause of an internally developing embryo. It would be interesting to enquire into the probable mechanism of the initiation and final release of this "diapause." The special development of certain glandular structures synchronising with the initiation and release of diapause makes it highly probable that these are under hormonal control. The following observations of the developmental cycle, accompanied by the enlargment or breakdown of the glandular structures concerned, are illuminating.

Stage 1. In *Lychas,* the development of the embryo up to the establishment of the germinal layers take place in the follicles outside the ovarian tube. The zygotes and blastoderms are surrounded by a thick dark pigmented shell or "chorion" (Fig. 1, A).



KEY TO LETTERING

A, large follicle with blastoderm surrounded by chorion.
A1, egg with blastoderm sinking into the ovarian tube.
A2, "empty chorion" from which the embryonic rudiment has passed out. Develops into corpus luteum 2, forming the embryonic feeding mechanism.
B, small follicles with eggs (earlier).
C, cells at the base of the follicle become glandular corpus luteum 1.
D, the cast off chorion and stalk—corpus luteum 3.
E, embryo.
Mg, midgut of the embryo.
St, stalk of the feeding mechanism connected with the embryonic gut.

FIGURE 1. Part of the ovarian tube (early) bearing follicles enclosing blastoderms or eggs.

FIGURE 2. A follicle with blastoderm sinking into the ovarian tube (A1), causing the part of the ovarian tube to bulge a little. At the site of the insinking (C, C) the outer cells begin to enlarge and become glandular.

FIGURE 3. The embryonic rudiment (E) is pushed into the ovarian tube from the ruptured "chorion" (A2), which is at the same time pushed upwards, everting the invaginated outer layer at the site of the insinking of the egg. The cells of this portion (C) have now become enlarged and glandular. At this stage they form button-like thickenings on the swollen parts of the ovarian tube in which are the developing embryos.

FIGURE 4. The upward movement of the chorion (A2) continues. It pushes itself out of the inner layer and projects out, covered over by the everted glandular cells of the follicle (C).

Stage 2. After extrusion of a brood, these follicular embryos surrounded by the "chorion," which appears to be developed out of follicular cells, sink into the ovarian tube (Fig. 2, A1). The site of the insinking marking the base of the follicle soon develops a spongy texture and becomes glandular (C in the figures). Soon this assumes the form of a button-like papilla with enlarged glandular cells. This structure may be regarded as *corpus luteum* 1. The term "corpus luteum" has been used by Laurie (1890) for the empty follicle in *Euscorpius* after the embryo has passed into the ovarian tube for further development. He surmised that the hormone from this corpus luteum prevents the development of new embryos. Here, however, it is not one structure that develops secretory properties in connection with the developmental cycle, but at least three different parts of follicular origin serve this function; these structures are here tentatively called corpus luteum 1, 2 and 3.

Stage 3. In subsequent development when the "embryonic rudiment" is passed out of the "chorion" into the ovarian tube, the partially empty "chorion" is pushed upwards through the spongy structure, that has been termed above corpus luteum 1, and breaking through it, gets exposed in the body cavity as a tiny rounded dark brown body, A2, retaining connection with the gut of the developing embryo (Fig. 5). This body with its stalk (St.) develops into the queer "feeding mechanism" which has been described elsewhere (Mathew, 1960). This structure, which also is of follicular origin, has been regarded as a corpus luteum and may be distinguished as corpus luteum 2 (A2). When this structure is fully formed, corpus luteum 1 is seen to form a spongy globular mass surrounding its stalk (Fig. 5. C.). As the embryo grows, it is seen to be fed with the rich secretion of the feeding mechanism, directly passed into the midgut of the embryo.

Stage 4. When the growth of the embryo is completed and it is about to be extruded, the connection with the midgut is constricted off where it joins the midgut and the whole stalk, tipped with the dark globular "chorion," slips off and drops into the mother's body cavity during parturition (Fig. 6).

Stage 5. Post-partum changes. The discarded feeding mechanism with its stalk (D), instead of immediately degenerating, develops into a club-shaped glandular organ, apparently actively functioning for some time. This may be called corpus luteum 3. Soon after, however, this structure begins to atrophy, undergoing histolysis.

DISCUSSION

From the above observations, it will be seen that corpus luteum 1 is formed immediately after the developing embryos have sunk into the ovarian tube. No more of the embryos sink in after this and so it is suggested that its secretion has the property of inhibiting further development of the follicular embryos: they remain outside the ovarian tube without sinking in and their growth appears to be arrested. In other words, they are in a state of "diapause" in which they remain till the developing embryos are extruded.

FIGURE 5. Finally the chorion (A2) breaks through the ovarian wall completely and projects out freely; the glandular cells (C) form a collar around the stalk (St) connecting the chorion with the gut of the embryo.

FIGURE 6. When parturition takes place, the chorion (A2) with its stalk (St) is separated off and left in the body cavity. There they develop into club-shaped bodies (D).

After the extrusion of the young, the feeding mechanism, consisting of the globular "chorion" and its club-shaped stalk, are released from the ovarian wall and drop into the body cavity. These curious bodies rapidly develop into glandular structures of a third type, called above corpus luteum 3. These are really spent structures which have served their purpose and are to disintegrate normally. But instead of that, they develop into glandular structures functioning actively for a time. To the secretion of this we can attribute only one function—that of "reviving" the embryos from their "diapause" condition, for, soon after they are released into the body cavity, fresh external embryos begin to sink in and continue development. At the same time it is significant that the glandular cells of corpus luteum 1, whose secretion was suggested to prevent further insinking of embryos, have shrunk up and become almost indistinct.

Soon after the "diapausing" or "resting" embryos have started their further course of development, we find that these bodies, corpus luteum 3, break up and disappear.

Here we have a singular instance of embryonic diapause in an internally developing egg. In insects, embryonic diapause is fairly well known; it occurs more commonly at stages more advanced than the blastoderm stage. But all these eggs are outside and their "diapause" and later its termination appear to be controlled by the influences of the external environment (Lees, 1955). In this scorpion, however, diapause occurs at the blastoderm stage or earlier (?), but never later. Further development of the blastoderm can take place only after it sinks into the ovarian tube, and later development appears to be an uninterrupted process. Naturally, the blastoderm stage is the most suitable period for an arrest of growth.

It is possible to look upon diapause in *Lychas*, also, as controlled by the environment—the maternal body cavity in which development takes place. Naturally it has not to depend on a precarious external environment but is under the influence of a well regulated internal environment changing rhythmically in correspondence with a reproductive cycle.

SUMMARY

1. An unusual instance of embryonic diapause in a scorpion is described. Among the eggs that are fertilised at a time, only a few sink into the ovarian tube and continue to develop to maturity while the others remain in a state of arrested growth or diapause, until the extrusion of the first batch of embryos. Further insinking of the eggs appears to be prevented by the secretion of corpus luteum 1.

2. After the extrusion of the first batch of embryos the diapausing embryos get revived and sink into the ovarian tube to give rise to the next brood. The termination of the "diapause," also, appears to be in response to a secretion from another glandular structure, termed here corpus luteum 3. This incidentally explains how *Lychas* can produce two or three broods in succession without undergoing repeated copulation.

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